THE EFFECT OF POSTNATAL GROWTH RESTRICTION ON SERUM FATTY ACIDS AND LUNG FATTY ACID INTERACTING PROTEINS IN THE RAT

by

Cheri Macie Kuliaikaui Bantilan

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STATEMENT OF THESIS APPROVAL

The thesis of **Cheri Macie Kuliaikaui Bantilan**

has been approved by the following supervisory committee members:

Lisa Joss-Moore	, Chair	**03/10/17** Date Approved
Kristine Jordan	, Member	**03/10/17** Date Approved
Julie Metos	, Member	**03/10/17** Date Approved

and by **Scott Summers** , Chair/Dean of

the Department/College/School of **Nutrition and Integrative Physiology**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Despite advances in the medical field, bronchopulmonary dysplasia (BPD) remains a major concern for preterm infants. A hallmark characteristic of BPD is impaired alveolar formation. Contributing to impaired alveolar formation and the development of BPD is respiratory support as well as poor postnatal growth and inadequate nutrition. The contribution of respiratory support has been well characterized in the development of BPD; however, the role that postnatal growth restriction (PGR) plays in impaired alveolar formation and the development of BPD is less understood.

Our study focused on determining the downstream molecular mediators of altered essential long-chain fatty acids in alveolar formation, peroxisome proliferator activated receptor gamma (PPARγ), and fatty acid binding protein 4 (FABP4). We **hypothesize** that PGR decreases circulating essential fatty acids in the rat, in association with increased lung mRNA and protein levels of FABP4, and reduced levels of lung PPARγ protein.

To test this hypothesis, PGR was induced in our rat model using variation in litter size by cross-fostering newborn rat pups into rat dams with litter sizes of 16 (PGR) or 8 (control). Rat pup weights were measured every other day from birth to day of life 21 (d21). GC-MS was used to measure serum and lung fatty acid profiles at d21. RT-PCR was used to measure PPARγ, and FABP4 mRNA abundance in the lung at d21. Western

blotting was used to measure PPARγ, and FABP4 protein abundance in the lung at d21.

PGR reduced serum LA, AA, ALA, and DHA in male rat pups compared to sex-matched controls. PGR significantly decreased PPARγ protein abundance in male rat pups compared to sex-matched controls. PGR also increased FABP4 mRNA, and FABP4 protein abundance in male rat pups compared to sex-matched controls.

We conclude that PGR alters serum fatty acid profiles, as well as PPARγ, and FABP4 levels in a sex-divergent manner. The effects that PGR has on serum fatty acid profiles, PPARγ, and FABP4 may have a potential impact on the development of BPD. We speculate that PGR causes alterations in serum fatty acids, which inhibit nutrient responsive signaling pathways that are vital for alveolar formation.

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INTRODUCTION

Bronchopulmonary Dysplasia (BPD) is a serious and common consequence of preterm birth. BPD, the chronic lung disease of early infancy, affects 10,000 preterm infants (<37 weeks gestation) each year in the United States.[1] BPD results in high morbidity and mortality in the neonatal period, and predisposes survivors to long-term deficits in lung health. Male infants are more severely affected than female infants, with increased risk and severity of BPD.[2,3] A key feature of BPD is impaired alveolar formation.

Impaired alveolar formation in the context of BPD occurs in part because, in the human lung, alveolar formation begins before term birth, at approximately 28-32 weeks gestation. Preterm infants being cared for in the neonatal intensive care setting frequently receive respiratory support secondary to immature lungs. Prolonged, invasive mechanical ventilation with oxygen rich gas is well established to contribute to the development of BPD. Another contributor to the development of BPD and impaired alveolar formation is poor postnatal growth and inadequate nutrition.[4,5,6]

Inadequate postnatal growth and nutrition often accompany preterm birth, and lead to postnatal growth restriction (PGR).[7] In preterm infants, PGR results from severe caloric deficits secondary to feeding intolerance, critical illness, and clinically indicated fluid volume restriction.[5,7]

During development, adequate nutrition is vital to supplying appropriate nutrients for activation of nutrient responsive signaling pathways. Evidence for the importance of nutrition on alveolar integrity is provided through animal models. We previously demonstrated, in a preterm lamb model of evolving BPD, that impaired nutrition in the postnatal period contributed to impaired alveolar formation.[4] We also showed that in a rat model, prenatal growth restriction impaired alveolar formation.[8,9] Other studies that support the role of nutrition in alveolar integrity include calorically restricted adult mice that have suppression of cell replication in the lung and alveolar destruction.[10,11] Importantly, refeeding the calorically restricted adult mice with an ad libitum diet stimulates cell replication gene expression and alveolar regeneration.[10,11] Collectively, these findings suggest that alveolar simplification in BPD may be a result from inadequate nutrition, or deficiencies in specific macronutrients that are important for the activation of nutrient responsive signaling pathways.

One macronutrient important in alveolar formation and BPD is the essential long chain fatty acid. Essential fatty acids are not only good energy sources; they also regulate nutrient responsive signaling pathways and moderate inflammation. Linoleic Acid (LA) is an essential omega 6 fatty acid (ω6), and a precursor molecule for Arachidonic Acid (AA). ω6 fatty acids, particularly AA, play an important role in phospholipid structure, cell division, and signaling.[12] Though adequate amounts of AA are important for infant growth, excessive levels of LA are associated with inflammation in preterm lung development. Also important for infant growth and alveolar formation are the essential omega-3 (ω3) long chain fatty acids, α-linoleic acid (ALA), and its derivative Docosahexaenoic Acid (DHA). DHA is associated with fetal development including fetal

lung development.[4,8]

Preterm infants who develop BPD have altered circulating essential fatty acid profiles characterized by decreased circulating AA and decreased circulating DHA. Also altered in preterm infants who develop BPD is the ratio of LA to DHA, which is increased.[13] During the last trimester of pregnancy, the placenta facilitates an increased transfer of AA and DHA, while limiting the transfer of LA. When the last trimester of pregnancy is disrupted by preterm birth, AA and DHA levels become limited. Preterm infants must rely on external sources for essential fatty acids, thus LA, the primary lipid used in neonatal intensive care feeding practices, become excessive.

An essential fatty acid that has been extensively studied in the context of the lung is DHA. Shown to reduce inflammation in lung development of preterm infants, DHA is also a peroxisome proliferator activated receptor gamma (PPARγ) agonist.[3,14,15] Lung development depends on nutrient activation of the transcriptional regulator PPARγ, in part, by DHA.

PPARγ, a member of the PPAR subfamily of nuclear receptors, is critical for lung development. PPARγ signaling is nutrient responsive, and inadequate nutrition can lead to alveolar simplification via PPARγ signaling.[8] One important ligand for PPARγ is DHA, which has a higher affinity for PPARγ than other long chain fatty acids.[16] Ligand activation of PPARγ by DHA results in the transcription of downstream PPARγ target genes that facilitate alveolar formation. However, in order for DHA to interact with PPARγ, DHA must be transported into the nucleus via a fatty acid binding protein.

Fatty Acid Binding Proteins (FABP) is a large family of lipid binding proteins. Known originally as the adipocyte-FABP, FABP4 is expressed in adipocytes and more

recently has been detected in the lung parenchyma and in alveolar macrophages.[15] FABP4 is thought to regulate the PPARγ pathway.[15] FABP4 is a fatty acid chaperone, with a high affinity for long chain fatty acids.[17,18] FABP4 facilitates the transport of non-esterified fatty acids from lipid droplets to the nucleus. The fatty acids brought into the nucleus via FABP4 subsequently enhance PPARγ transcriptional activity.[17–19] The trafficking of long chain essential fatty acids into the nucleus is imperative for activation of PPARγ and alveolar formation.[19]

The interaction between FABP4 and PPARγ extends beyond shuttling of lipids into the nucleus. The FABP4 gene is a transcriptional target of PPARγ, and the FABP4 protein stimulates degradation of PPARγ by the 20S proteasome. In a negative feedback loop, PPARγ induces FABP4 mRNA expression. As FABP4 protein accumulates, it accelerates PPARγ degradation.[20]

Previous studies demonstrate that the combination of respiratory support and PGR affect lung development, particularly alveolar formation.[4] However, the isolated contribution of PGR to alveolar formation has not been examined. To understand the isolated contribution of postnatal nutrition to alveolar formation, we optimized a previously developed rat model of PGR. We showed that lungs of PGR rat pups differ from control in terms of both alveolar formation and lung function, with sex-divergent outcomes. Specifically, we showed that in female rat lungs, PGR resulted in thicker alveolar walls, but with increases in indices of lung maturation (secondary septa formation and radial alveolar count). In contrast, in male rat lung, PGR again resulted in increased alveolar wall thickness, but without a change in indices of lung maturation. Concomitant with the structural differences between PGR and control lungs, we

demonstrated that PGR decreased lung compliance in both female and male rat pups, and increased lung resistance, with greater effects in male rat lungs.

However, the effect of PGR on essential fatty acids, PPARγ, and FABP4 in the rat lung is unknown. We **hypothesize** that PGR decreases circulating essential fatty acids in the rat, in association with increased lung mRNA and protein levels of FABP4, and reduced levels of lung PPARγ protein.

METHODS

PGR was induced in Sprague Dawley rat pups using variation in litter size.[21] Newborn rat pups were cross-fostered to rat dams with litter sizes of 16 (PGR) or 8 (control), with equal numbers of female and male pups in each litter. Rat pup weight was measured every other day from birth to postnatal day 21 (d21). At d21, control and PGR rat offspring underwent euthanasia using a sodium pentobarbital overdose, and serum and lung tissue were collected and immediately flash frozen in liquid nitrogen. In this study, we examined 4 groups: PGR females, PGR males, control females, and control males. Each group consisted of a sample size of 6-10 non-sibling rat pups.

Gas Chromatography- Mass Spectrometry (GC-MS)

Serum and lung LA, AA, ALA, and DHA were quantified using GC-MS at the University of Utah Core facility. Frozen serum was transferred to the University of Utah Core facility to quantify relative amounts of fatty acids. Supelco's FAMES 37 component mixture was used as a reference standard. Serum fatty acids are expressed in ng/μL serum. For lung fatty acids, a volume of 1 mL of 3% HCl in MeOH containing Supelco's FAMES 37 was added to lung tissue samples. Lung samples were homogenized and centrifuged at 15,000 G for 10 minutes at 4 °C. Lung fatty acids are expressed in ng/mg lung tissue. Note, lung fatty acid measurements included only free or esterified fatty

acids, and not phospholipids.

Real-Time RT-PCR

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify levels of two PPARγ mRNA variants, PPARγ1 and PPARγ2, as well as FABP4 mRNA in lungs of rat pups (PGR and control). RNAeasy Mini Kit (Qiagen) was used to extract RNA from lung tissue samples. To generate cDNA, a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used. The following Assay-on demand primer/probe sets were used: PPARγ1 – Rn01492274_m1, PPARγ2 – Rn00440940_m1, FABP4 – Rn00670361_m1. GAPDH was used as an internal control. GAPDH primer and probe sequences; Forward: CAAGATGGTGAAGGTCGGTGT; Reverse: CAAGAGAAGGCAGCCCTGGT; Probe: GCGTCCGATACGGCCAAATCCG. QuantiStudio 12K Flex Real Time PCR system was used for RT-PCR amplification at the University of Utah Genomics Core Facility and for analyzing data. The comparative CT method was used to determine mRNA levels.[22]

Western Blotting

Western blots were used to quantify protein abundance of PPARγ, and FABP4 in the rat lung. Rat lung tissue was crushed using a mortar and pestle with liquid nitrogen. A RIPA buffer and protein inhibitor mixture (Roche-Complete Mini) was used to isolate total protein. Pierce BCA protein assay kit (ThermoScientific) was used to quantify

protein abundance. Protein samples were stored at -80° C until use. A total of 10 μg of protein homogenate was loaded on a 10% Bis-Tris Polyacrylamid Midi Gels (Novex by Life Technologies) to analyze PPARγ protein abundance. A total of 15 μg of protein homogenate was loaded on a 4-12% Bis-Tris Polyacrylamide Midi Gels (Novex by Life Technologies) to analyze FABP4 protein abundance. The following primary antibodies were used: PPARγ (H-100, Santa Cruz Biotechnology), FABP4 (12802-1-AP, ProteinTech), FABP4 (RP1085, Boster), and GAPDH (14C10, Cell Signaling Technology). All antibodies were identified with Western Lighting enhanced chemiluminescence and quantified using an Image Station 2000R (Eastman Kodak).

Statistical Analysis

Female and male rats were considered separate groups. Data are presented as mean + standard deviation, for a sample size of n= 6-10 per group. Non-parametric tests were used to detect statistically significant differences. For comparisons between all groups, ANOVA with Fisher's Least-Significant-Difference test was used. For pair-wise comparisons, Mann-Whitney U test was used. Analysis was performed using the Statview statistical program. Statistical significance was defined as $p \leq 0.05$ for all analyses.

RESULTS

PGR and Control Body Weights

PGR affects both female and male rat pups. PGR female rat pups weigh significantly less than their sex-matched control by postnatal d5 and continue to weigh significantly less through postnatal d21 (Figure 1A). Similarly, PGR male rat pups weigh significantly less than their sex-matched control by postnatal d5 and continue to weigh significantly less through postnatal d21 (Figure 1B).

Serum and Lung Fatty Acid Levels

In order to understand the effects of PGR on fatty acid levels in the serum and in the lung, we quantified LA, AA, ALA, and DHA, as well as LA:DHA ratios in female and male, Control, and PGR rat pups. We also assessed whether there was a baseline sex-difference in serum or lung fatty acid levels in the control rats.

For LA, levels were similar between control female and control male rat pups in both the serum and the lung. In serum, PGR increased LA levels in female rat pups compared to female controls ($p < 0.05$, $n = 6$) (Figure 2A). In contrast, PGR decreased LA levels in male rat pups compared to male controls ($p < 0.01$, $n = 6$) (Figure 2A). In lung, PGR did not affect LA levels in female or male groups (Figure 2B).

For AA, control male rat pups had significantly higher serum AA than control

female rat pups ($p < 0.01$, $n = 6$); however, lung AA levels were similar between male and female rats. In serum, PGR increased serum AA in female rat pups compared to female controls ($p < 0.05$, $n = 6$) (Figure 3A). Again, in contrast, PGR decreased AA in male rat pups compared to male controls ($p < 0.01$, $n = 6$) (Figure 3A). In lung, PGR did not affect lung AA in female or male rat pups (Figure 3B).

For ALA, levels were similar between control female and control male rat pups in both the serum and the lung (Figure 4A). In serum, PGR did not affect ALA levels in females (Figure 4A). However, PGR decreased ALA in male serum compared to male controls ($p < 0.05$, $n = 6$) (Figure 4A). In lung, PGR did not affect ALA levels in female or male rat pups (Figure 4B).

For DHA, control male rat pups had significantly higher serum DHA than control female rat pups ($p < 0.05$, $n = 6$) (Figure 5A), while male rat pups had lower lung DHA than female rat pups ($p < 0.05$, $n = 6$) (Figure 5B). In serum, PGR did not affect DHA levels in female rat pups. In contrast, PGR decreased serum DHA in male rat pups compared to male controls ($p < 0.01$, $n = 6$)(Figure 5A). In lung, PGR decreased DHA levels in female rat pups compared to female controls ($p < 0.01$, $n = 6$). In contrast, PGR did not affect lung DHA in male rat pups (Figure 5B).

For LA:DHA ratios, levels were similar between female control and male control rat pups both in the serum and in the lung. In serum, PGR did not affect LA:DHA ratios in female rat pups. However, PGR significantly increased LA:DHA ratios in male rat pups compared to controls ($p < 0.05$, $n = 6$) (Figure 6A). In lung, PGR did not affect LA:DHA ratios in female or male rat pups (Figure 6B).

PPARγ and FABP4 mRNA Levels

PPARγ1 and PPARγ2 mRNA transcript levels were not different between control female and control male rat lung (Figure 7A). Similarly, PGR did not alter lung levels of PPARγ1, or PPARγ2 mRNA transcript in female or male rat pups (Figure 7A, 7B).

FABP4 mRNA transcript levels were also not different between control female and control male rat lung (Figure 8). PGR did not alter lung levels of FABP4 mRNA transcript in females compared to female control (Figure 8). In contrast, PGR increased FABP4 mRNA transcript levels in male rat lung compared to male control ($p < 0.01$, $n = 6$) (Figure 8).

PPARγ and FABP4 Protein Abundance

PPARγ protein abundance is higher in control male rat lung than in control female rat lung ($p < 0.01$, $n = 6$) (Figure 9). FABP4 protein abundance was not different between female control and male control rat lung (Figure 10A, Figure 10B). PGR did not alter PPARγ protein abundance in female rat lung compared to female control. However, PGR decreased PPARγ protein abundance in male rat lung compared to male control ($p < 0.01$, $n = 6$) (Figure 9). Similarly, PGR did not affect FABP4 protein abundance in female rat pups compared to female control (Figure 10A). However, PGR significantly increased FABP4 protein abundance in male rat pups compared to male control ($p \leq 0.05$, $n = 6$) (Figure 10B).

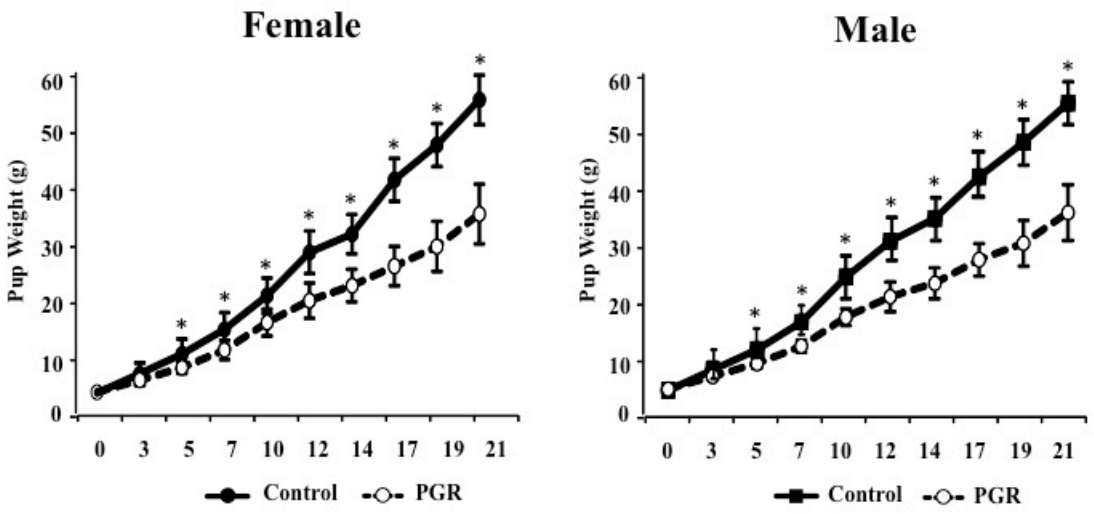

Figure 1 Growth Trajectory of Female and Male, Control and PGR Rat Pups: (A) Weight of female control and PGR rat pups, and (B) weight of male control and PGR rat pups. PGR female and male body weight is significantly less than sex-matched control by postnatal d5 and continued to be significantly less through postnatal d21.

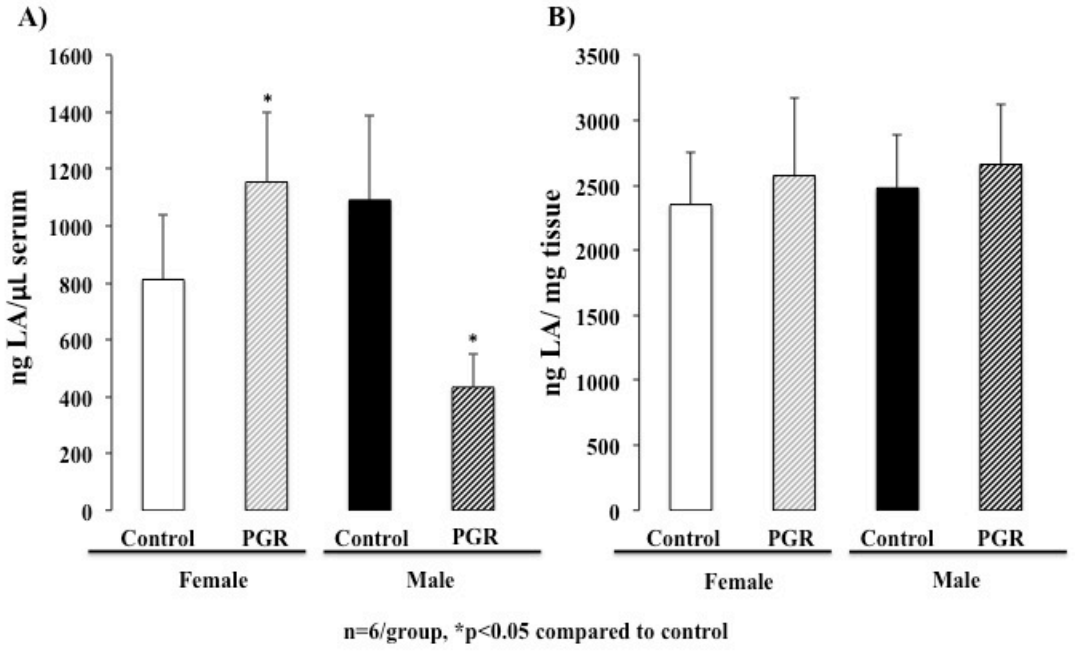

Figure 2 Serum and Lung LA Levels: (A) ng LA/ µL serum in female and male, control and PGR rat pups and, (B) ng LA/mg tissue in female and male control and PGR rat pups. PGR significantly increases serum LA in PGR females and significantly decreases serum LA in PGR males. No significant changes were detected in the lung.

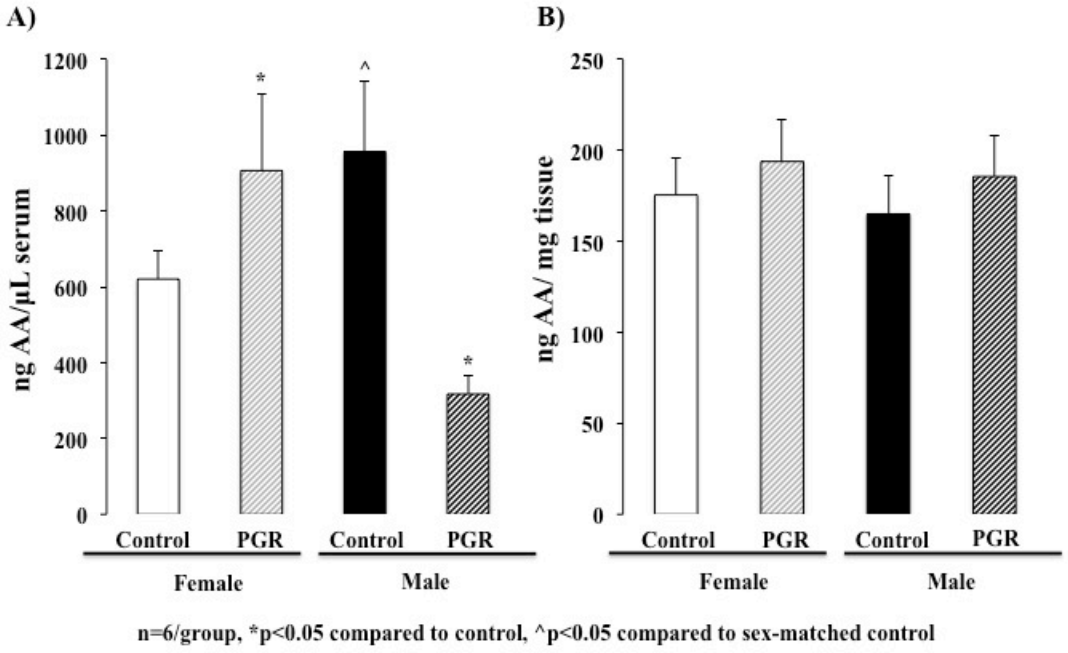

Figure 3 Serum and Lung AA Levels: (A) ng AA/ µL serum in female and male, control and PGR rat pups, and (B) ng AA/mg tissue in female and male control and PGR rat pups. Male controls have increased serum AA compared to female controls. PGR increases serum AA in females, and decreases AA in males. No significant changes were detected in the lung.

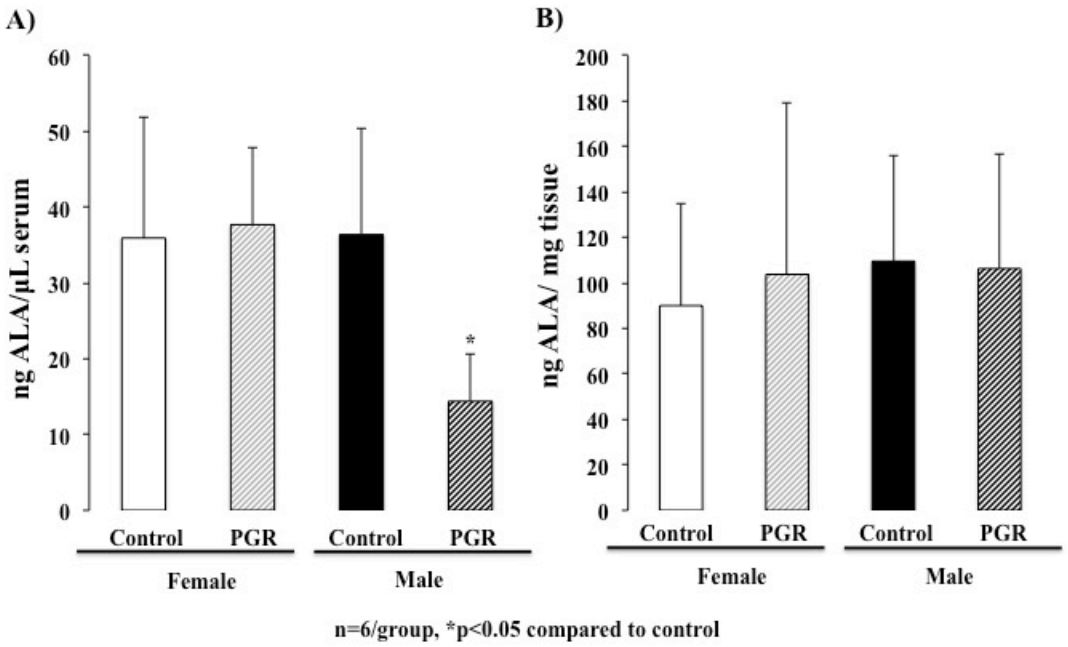

Figure 4 Serum and Lung ALA Levels: (A) ng ALA/ µL serum in female and male, control and PGR rat pups and, (B) ng ALA/mg tissue in female and male control and PGR rat pups. Male controls have no change in serum ALA compared to female controls. PGR has no effect on female rat pups compared to sex-matched control, but decreases serum ALA in males. No significant changes were detected in the lung.

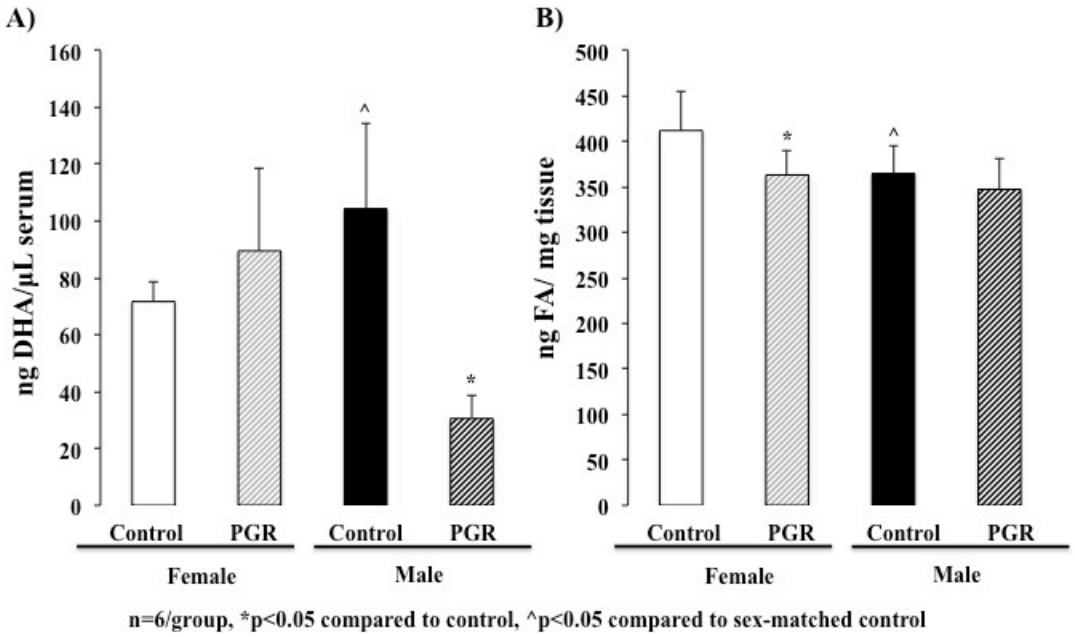

Figure 5 Serum and Lung DHA Levels: (A) ng DHA/ µL serum in female and male, control and PGR rat pups, and (B) ng DHA/mg tissue in female and male control and PGR rat pups. Male controls have significantly higher DHA compared to female controls. PGR decreases serum DHA in males, but has no affect on females. In lung, male controls have significantly lower lung DHA compared to female controls. PGR decreases lung DHA in females, but not in males.

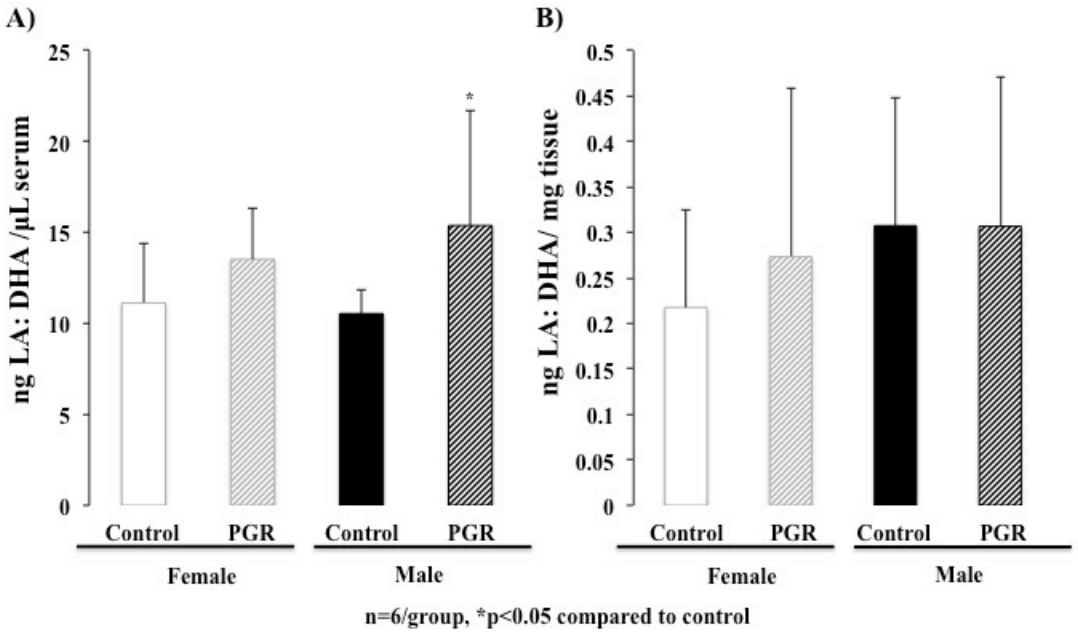

Figure 6 Serum and Lung LA:DHA Ratios: (A) ng LA:DHA/ µL serum in female and male, control and PGR rat pups, and (B) ng LA:DHA/mg tissue in female and male control and PGR rat pups. PGR increases serum LA:DHA in males, but has no effect on females. No significant changes were detected in the lung.

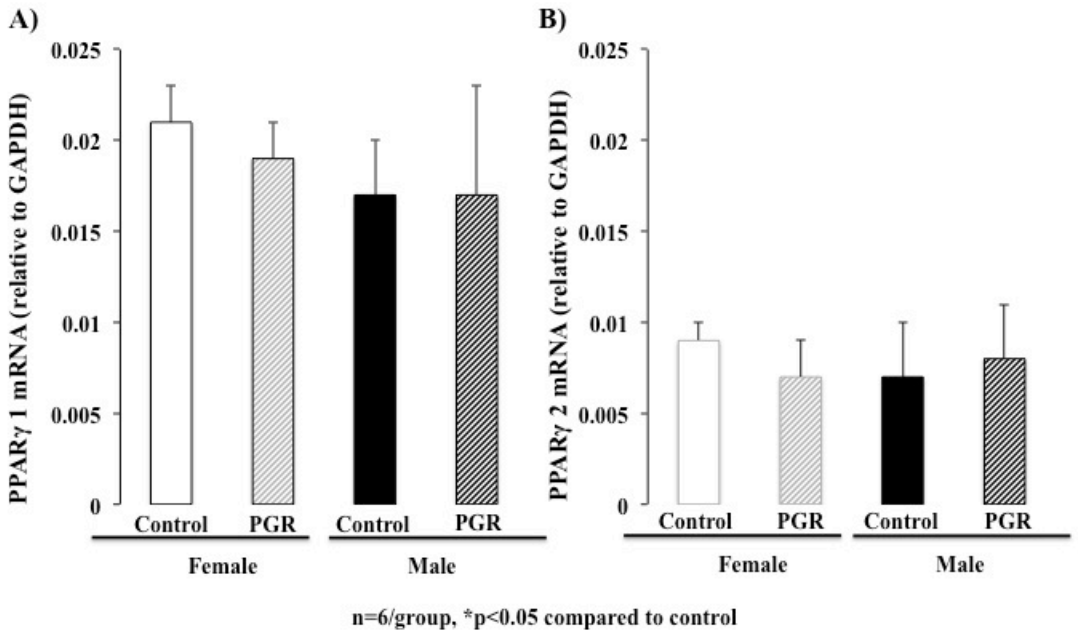

Figure 7 PPARγ1 and PPARγ2 mRNA Transcript Levels: PGR had no effect on (A) PPARγ1 mRNA transcript in female and male rat pups, or (B) PPARγ2 mRNA transcript levels.

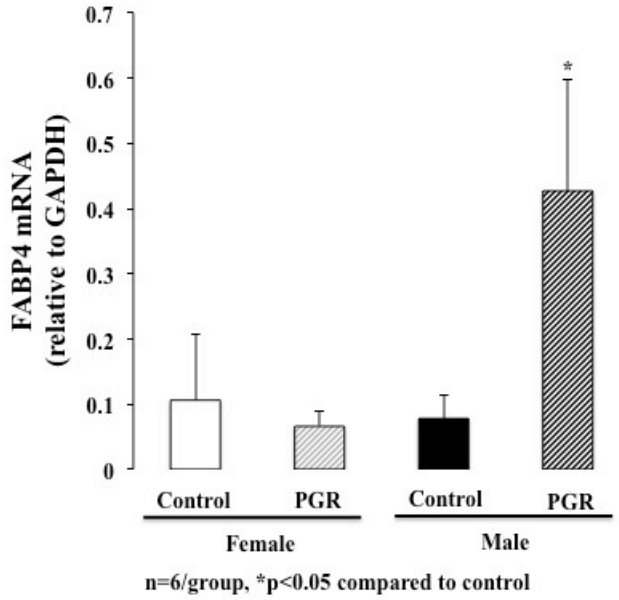

Figure 8 FABP4 mRNA Transcript Levels: PGR had no effect on FABP4 mRNA transcript levels in female rat pups. PGR significantly increased FABP4 mRNA transcript levels in male rat pups.

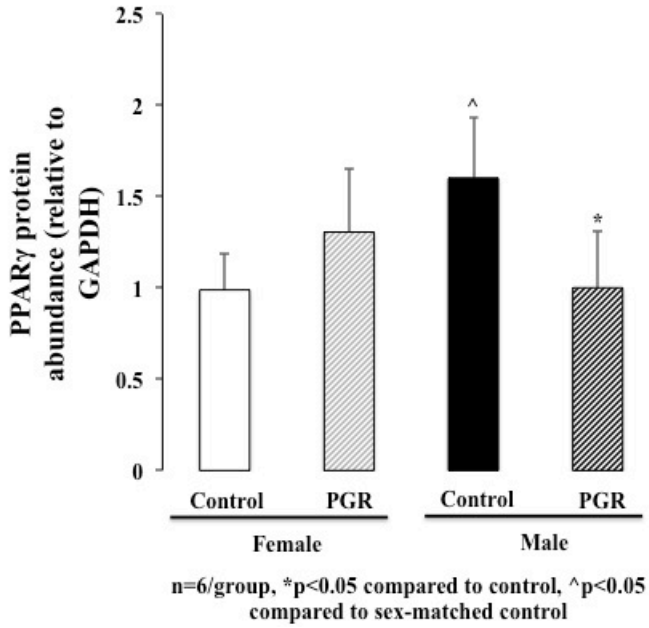

Figure 9 PPARγ Protein Abundance: Male controls have significantly higher PPARγ protein abundance compared to female controls. PGR decreases PPARγ protein abundance in males, but has no effect on females.

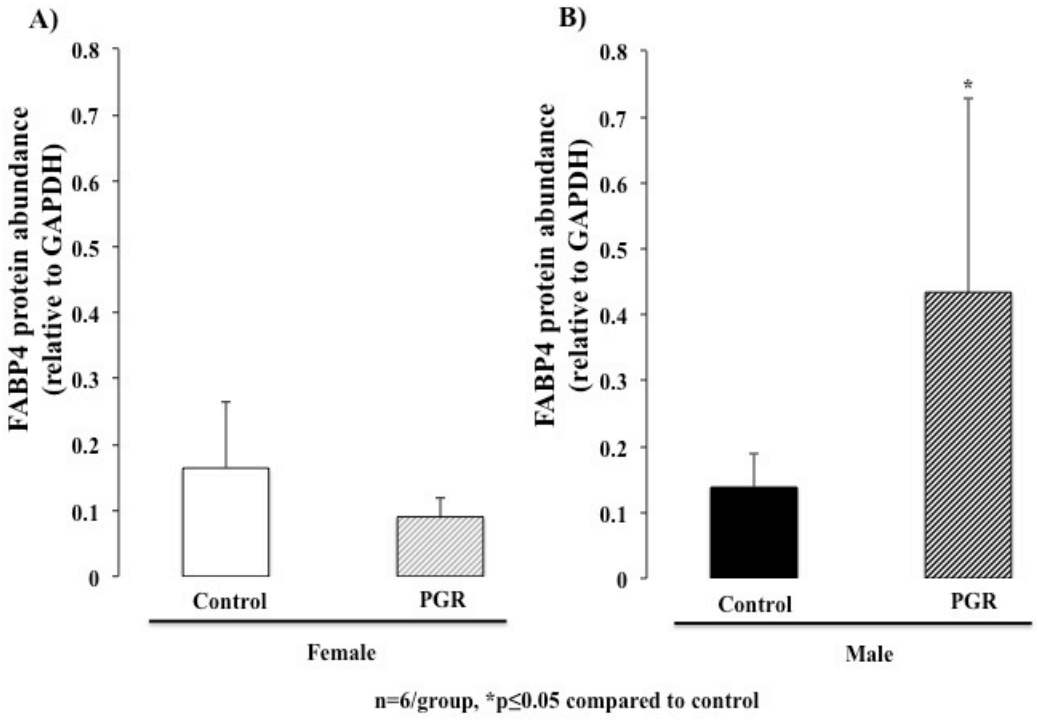

Figure 10 FABP4 Protein Abundance: PGR has no effect on (A) FABP4 protein abundance in female rat pups. PGR significantly increases (B) FABP4 protein abundance in male rat pups.

DISCUSSION

In our study, we demonstrated that, in the rat, PGR causes sex-divergent changes in serum fatty acids, as well as lung expression of PPARγ and FABP4. Combined with our previous data showing sex-divergent alterations in alveolar formation, and lung function in PGR rat pups, these data suggest that altered fatty acid signaling may contribute to sex-divergent lung outcomes. PGR-induced changes to fatty acid signaling may be manipulated with dietary fatty acid supplementation. Therefore, these data will inform subsequent studies aimed at developing a targeted fatty acid-based intervention to improve lung outcomes in PGR.

In our study, PGR significantly decreased serum levels of LA, AA, ALA, and DHA, but only in male rat pups, with minimal effects in female rat pups. In addition, the ratio of LA:DHA was increased in male, but not female, rat pups. Similar findings were observed in a study by Martin et al., in which preterm infants who developed BPD had a fatty acid profile of decreased AA, DHA, and increased LA: DHA ratio.[13] These alterations in serum fatty acid profiles have two important implications. First, the profile observed in our male rat pups suggests that PGR may have an impact on alveolar formation by inhibiting the activation of nutrient signaling pathways. Potentially, a similar mechanism contributes to poor lung outcomes in human preterm infants, who also tend to experience PGR. Secondly, the observed changes in serum fatty acid profiles are

not related to intake, as both female and male rat pups received rat milk from the same rat dams. Therefore, sex-specific fatty acid profiles may be the result of sex-specific fatty acid metabolism within the liver.[23] In a study by Ryan et al., in which preterm infants were randomly assigned to a control formula or a formula supplemented with DHA, preterm male infants, but not preterm female infants, had a significant negative effect on growth and body composition.[24] These results in combination with other studies imply that sex hormones may influence fatty acid metabolism through the enzymatic synthesis of long chain fatty acids. Another possible contributing factor to sex-specific differences in fatty acid uptake is different tissues, including adipose tissue.[25] Preterm infants are more prone to develop increased adipose tissue, especially intra-abdominal adiposity, as compared to term-born infants.[26] In a study by Thomas et al., ex-preterm, adult men were susceptible to accumulating excess internal adipose tissue, specifically in the abdominal area.[27] Lack of change in fatty acid profiles within non-phospholipid space within the lung, however, suggest that this particular compartment is not contributing to sequestering of lipids in a sex-divergent manner.

Historically, serum fatty acid levels have been used as biomarkers for lung diseases including lung cancer, chronic lung disease, and BPD.[13,28] Our observations are consistent with the use of serum fatty acid profiles as a marker of lung outcomes, despite these profiles not reflecting lung fatty acid profiles.

The involvement of PPARγ signaling in lung developmental outcomes is well established. Required for lung development and lung vascular integrity, PPARγ is necessary for epithelial-mesenchymal interactions.[29–31] Altered lung development, along with decreased expression of elastin, was a result of lung PPARγ-knock out in mice.

Additionally, prenatal restricted rat pups have demonstrated impaired alveolar formation, decreased PPARγ, and altered lung function compared to non-growth-restricted rat pups.[8,9] Consistent with these observations, in our model, PGR reduced PPARγ lung protein abundance in male rat pups. Given our previous observations of thicker airspace walls and altered lung mechanics in male rats following PGR, we speculate that decreased PPARγ may be contributing to lung outcomes in male rats, potentially by reducing expression of target genes critical for lung development.[30]

Our study also provides novel insight into potential mechanisms by which PPARγ protein may be reduced in the lung of male PGR rat pups. Previous studies demonstrated a negative feedback loop between FABP4 and PPARγ, in which FABP4 negatively regulates PPARγ.[20] The authors of this study demonstrated that FABP4 accelerates proteasomal degradation of PPARγ.[20] Our findings of increased FABP4 protein abundance in PGR male rat pups, in the context of reduced PPARγ, are consistent with this mechanism. Ghelfi et al. showed in their baboon model of BPD increased levels of FABP4 mRNA transcript levels and increased FABP4 protein abundance in BPD tissues compared to their controls.[15] FABP4 was also detected in human lung samples of BPD in the same study. Ongoing studies are evaluating PPARγ ubiquitination and targeting to the proteasome in PGR rat lungs.

An important part of the negative feedback loop between PPARγ and FABP4 is the regulation of FABP4 transcription by PPARγ.[20] Again our data demonstrating increased FABP4 mRNA transcript in PGR males, as well as FABP4 protein, support the presence of a negative feedback loop involving PPARγ and FABP4. The mechanism by which only male rat pups are affected is not known. However, given the role of FABP4

as a fatty acid chaperone with the role of transporting fatty acids into the nucleus to activate PPARγ, we speculate that up-regulation of FABP4 may be a compensatory in the face of reduced serum essential fatty acids in male rat pups.[19,20]

Our study is not without limitations. First, we did not examine the composition of the rat milk being produced by control and PGR rat pups. Previous studies do suggest that varying rat litter sizes influence milk composition, and therefore nutrient intake of rat pups.[32] Second, we did not assess the phospholipid compartment in the lung. It is possible that sequestering of circulating lipids in male rat lungs occurs at the level of the phospholipid membrane.[33,34] Ongoing studies are evaluating the fatty acid profile of this compartment. Finally, our study is associative and does not confirm a cause-and-effect relationship between serum lipids, PPARγ, or FABP4 and lung outcomes. Ongoing studies in the lab are supplementing rat dams with diets containing DHA and/or AA in the context of PGR to determine if lung outcomes can be improved by restoring lipid profiles.

In conclusion, our study demonstrates that PGR alters serum fatty acid profiles, as well as PPARγ, and FABP4 levels, all of which may have a potential impact on alveolar formation and the development of BPD. The sex-divergent changes that we observed are consistent with previous studies which have found that male infants are more severely affected than female infants, with increased risk and severity of BPD.[2,3] We speculate that PGR-induced alterations in serum fatty acids impede nutrient activated pathways vital for alveolar formation. Our results emphasize the important role that postnatal nutrition has on a molecular pathway that is integral for alveolar formation and the prevention of BPD.

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